Tamoxifen metabolism and pharmacogenetics in breast cancer

Dezentjé, V.O.

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General discussion

Vincent O. Dezentjé

INTRODUCTION

Tamoxifen, originally described as an antifertility agent in rats, is a selective estrogen receptor modulator (SERM), which is now widely used for the treatment of breast cancer since its drug approval in the 1970s.[1] Noteworthy, tamoxifen was one of the first targeted agents in oncology, selectively blocking the estrogen receptor in breast cancer cells. Nonetheless, not all patients with hormone receptor positive breast cancer benefit from tamoxifen treatment. In metastatic breast cancer approximately 50% of the patients do not respond to tamoxifen and in early breast cancer one third of the patients will have a disease recurrence despite five years of tamoxifen therapy.[2-5] Thus, a substantial number of patients will be potentially exposed to tamoxifen's side effects without any gain. A better selection of patients who will likely respond to tamoxifen or develop relevant side effects will greatly improve hormonal therapy in breast cancer.[6] We hypothesized that the degree of biotransformation from the pro-drug tamoxifen to its active metabolites endoxifen and 4-hydroxytamoxifen will be predictive of both tamoxifen efficacy and side effects. Endoxifen is considered the most important metabolite and the liver cytochrome P450 2D6 (CYP2D6) enzyme is mainly responsible for its formation.[7-11] In this thesis variation in tamoxifen metabolism is studied and described in relation to tamoxifen efficacy and side effects with a focus on CYP2D6 activity and endoxifen.

Potential bias and effect modification

The many publications reporting on the correlation between CYP2D6 genotype and tamoxifen efficacy have provided conflicting data.[12-18] However, all these studies, by virtue of their retrospective nature, were prone to limitations. Preferably, such pharmacogenetic analyses are performed on patients who were enrolled in prospective studies ensuring good documentation of patient and tumor characteristics and clinical endpoints. Nonetheless, evidence of an association between CYP2D6 genotype and well-known prognostic patient or tumor characteristics, such as age, tumor size and nodal status is lacking and biologically not plausible. Therefore, these characteristics will unlikely cause bias as Mendelian randomization will balance these prognostic characteristics between the different CYP2D6 genotypes (**chapter 2**). Two important factors however may have caused effect modification in these analyses. Both concomitant CYP2D6 inhibitor use and tamoxifen adherence may be associated with tamoxifen efficacy, but also with the CYP2D6 genotype. A genotype encoding a normally active CYP2D6 enzyme (extensive metabolizer) may lead to more side effects and consequently a reduction in tamoxifen adherence.[13, 19] Paradoxically, this may lead to a worse

clinical outcome despite the normal biotransformation to endoxifen. Strong CYP2D6 inhibitors, such as paroxetine, are frequently prescribed in breast cancer patients to treat depression and hot flashes, a common tamoxifen side effect.[20] Patients with a CYP2D6 extensive metabolizer (EM) genotype may experience more hot flashes than patients with a CYP2D6 poor (PM) or intermediate metabolizer (IM) genotype and will consequently use more CYP2D6 inhibitors.[13, 19] Strong CYP2D6 inhibitors have the ability to phenocopy a PM phenotype in patients with an EM genotype, resulting in low endoxifen levels.[21] Thus, more side effects in EMs resulting in more frequent CYP2D6 inhibitor use and decreased tamoxifen adherence might mask a difference in tamoxifen efficacy between PMs and EMs. Therefore, the most optimal pharmacogenetic study on CYP2D6 and tamoxifen is a prospective study in which CYP2D6 genotype is comprehensively analyzed with concomitant CYP2D6 inhibitor use and tamoxifen adherence.

Laboratory methods

In our studies good methodology to genotype and measure endoxifen levels is imperative in order to retrieve reliable and comparable data. In the large clinical trials on adjuvant tamoxifen such as ATAC, BIG1-98 and TEAM valuable clinical data have been collected, but only formalin fixed paraffin embedded (FFPE) tumor containing tissue was available for genotyping.[16, 17] DNA derived from FFPE tissue is often fragmented and crosslinked and therefore difficult to genotype. We have reported a reliable method of pre-amplification of DNA in order to optimize genotyping results when DNA from FFPE tissue is used (**chapter 3**). This method was used in our pharmacogenetic studies in **chapters 5 and 6**. For the genetic variants that were tested in this study, call rates ranging from 84 to 100% were achieved while using a minimum amount of DNA. DNA was however retrieved from tissue blocks containing tumor, while the primary interest is the germline CYP2D6 genotype. Thus, in order to obtain relevant data, the genotype tested in the tumor block should be similar to the germline genotype. A recent publication that reported no association between the CYP2D6 genotype and tamoxifen efficacy was heavily criticized for using DNA derived from tumor blocks.[16] Loss of heterozygosity (LOH) events on chromosome 22q have been reported in up to 25% of hormone receptor positive breast tumors.[22, 23] Because the chromosomal locus of CYP2D6 is 22q13, LOH in tumor tissue may have caused false CYP2D6 genotype assignment. In our studies, we accounted for the potential influence of LOH in tumor tissue by performing an additional microsatellite analysis (**chapters 5 and 6**). To our best knowledge, these are the first reports of such an analysis resulting in reliable CYP2D6 genotypes despite the use of DNA from FFPE tumor containing tissue. We excluded the influence of LOH on genotype assignment in our analyses for nearly all patients by demonstrating

heterozygosity of one or more microsatellites flanking the CYP2D6 gene. A plausible explanation for this finding is that tissue blocks which contain tumor also contain a significant amount of normal tissue. Therefore, genotyping was, in fact, mainly performed on germline DNA.

In studies aiming to correlate endoxifen levels with CYP2D6 genotype or phenotype and tamoxifen efficacy, a reliable detection and quantification of endoxifen serum or plasma concentrations is imperative. Previously, endoxifen and 4-hydroxytamoxifen concentrations were overestimated by a factor 2 to 3 because chromatographic peaks of 4'-endoxifen and 4'-hydroxytamoxifen were overlapping endoxifen and 4-hydroxytamoxifen peaks respectively.[24] In **chapter 4** we described an LC-MS/MS method resulting in chromatographic separation of 4'-endoxifen and 4'-hydroxytamoxifen. This method accurately detected and quantified tamoxifen, N-desmethyltamoxifen, 4-hydroxytamoxifen and endoxifen and was applied to the studies described in **chapters 8 to 11**.

CYP2D6 activity and clinical outcome

In **chapters 5 and 6** the CYP2D6 genotypes and predicted phenotypes were related to disease free survival (DFS-t) and the occurrence of hot flashes during tamoxifen use in a Dutch patient cohort of the Tamoxifen Exemestane Adjuvant Multinational (TEAM) trial. We demonstrated that in postmenopausal early breast cancer patients treated with adjuvant tamoxifen followed by exemestane neither the CYP2D6 genotype nor the predicted phenotype was associated with DFS-t (**chapter 5**). The predicted CYP2D6 phenotype was based on genotyping five CYP2D6 alleles and concomitant CYP2D6 inhibitor use. In the analysis we used disease free survival during tamoxifen use as the primary endpoint: patients were censored at the time of tamoxifen discontinuation. Thus, we avoided effect modification by subsequent aromatase inhibitor use and also accounted for the potential interaction between CYP2D6 phenotype, side effects and consequently early tamoxifen discontinuation. The CYP2D6 genotype and the predicted phenotype were also not associated with the complete disease free survival after five years of follow-up, including the period of exemestane use.

In **chapter 6** we described the correlation between the CYP2D6 genotypes or predicted phenotypes and hot flashes as adverse event during tamoxifen therapy. In a previous report EMs experienced more moderate and severe hot flashes than PMs. In our study however, the CYP2D6 genotypes and predicted phenotypes were not associated with the occurrence of hot flashes during tamoxifen use.

Concomitant use of a CYP2D6 inhibitor may impair tamoxifen efficacy. Retrospective studies in which the association between CYP2D6 genotype and tamoxifen efficacy was studied often lack complete

information on co-medication or tamoxifen adherence.[12, 13, 15-18] In the pharmaco-epidemiological study described in **chapter 7** a pharmacy database, a pathology database and the Dutch Medical Register, containing discharge diagnoses after hospital admission were all linked. Information on the primary breast cancer diagnosis, breast cancer recurrence based on pathology or hospital admission data and nearly complete prescription data of tamoxifen and CYP2D6 inhibitors were available.[25, 26] In multivariable analysis we were able to adjust for many prognostic factors including tamoxifen adherence in contrast to other studies. In this study we did not find an association between concomitant CYP2D6 inhibitor use and breast cancer recurrence among patients treated with adjuvant tamoxifen. However, we were not able to adjust for CYP2D6 genotype or adverse effects that could lead to confounding bias or effect modification. On the basis of our study results, there is insufficient evidence to withhold CYP2D6 inhibitors from patients during tamoxifen therapy. Nonetheless, because similar other studies reported an increased breast cancer risk of concomitant CYP2D6 inhibitor use, we suggest using non-CYP2D6 inhibitors whenever possible while additional studies are awaited.[27-29]

An explanation for our negative pharmacogenetic association studies might be that the predicted CYP2D6 phenotype insufficiently predicts endoxifen serum concentrations. Furthermore, the CYP2D6 genotype or phenotype is merely a proxy for endoxifen concentration.[21, 30] If endoxifen levels are not predictive of tamoxifen efficacy and estrogen dependant side effects, the CYP2D6 genotype or predicted phenotype will not either. Finally, a potential explanation for our negative findings is that our studies were not statistically powered to find a relatively small difference between the CYP2D6 poor and extensive metabolizers or CYP2D6 inhibitor users and non-users.[23]

Endoxifen

In **chapter 11** we described the outline of the prospective CYPTAM study (NTR1509) that aims to relate CYP2D6 predicted phenotypes and endoxifen serum concentrations to disease free survival (primary aim), relapse free and overall survival (secondary aims) in tamoxifen treated early breast cancer patients. The first results of an additional analysis in which the CYP2D6 predicted phenotypes are related to steady state serum endoxifen trough levels are presented. The CYP2D6 phenotype predicted by a broad analysis of 33 CYP2D6 alleles using the Amplichip CYP450 test (Roche Diagnostics, Indianapolis, US) was significantly associated with (log-transformed) endoxifen serum concentrations. The 43% explained variance was higher than the 23% previously reported, although CYP2D6 inhibitor use was not yet accounted for in our current analysis (but will be in the final analysis).[30] Additionally, we were able to identify the heterozygous EM (hetEM) phenotype as

a distinct phenotype with a different mean endoxifen level than the other phenotypes. Nonetheless, the limited explained variance of endoxifen serum concentration by the CYP2D6 phenotype predicted by genotype stresses the need for a test that is more predictive of endoxifen levels. In **chapter 10** a new 13C-dextrometorphan breath test (DM-BT) is described for phenotyping CYP2D6. The CYP2D6 phenotype determined by the DM-BT explained 47.5% of the variation in (log-transformed) serum steady-state endoxifen levels, which is only a small improvement compared to the CYP2D6 phenotype predicted by genotype. The DM-BT might be useful along with CYP2D6 genotyping as it also accounts for concomitant CYP2D6 inhibitor use and potentially other environmental and epigenetic factors that influence CYP2D6 activity. An advantage of both CYP2D6 genotyping and the DM-BT is that these tests can be performed prior to tamoxifen treatment as opposed to measuring endoxifen concentrations. Nonetheless, endoxifen serum or plasma concentrations may better predict tamoxifen efficacy.

FUTURE RESEARCH PERSPECTIVES

The hypothesis that CYP2D6 activity predicts tamoxifen efficacy is based on the assumption that endoxifen is the most active tamoxifen metabolite in tamoxifen treated breast cancer patients. Patients with decreased or absent CYP2D6 activity have lower endoxifen blood levels, but whether these levels are low enough to impair efficacy is uncertain. The data from retrospective studies addressing the relation between CYP2D6 genotype, predicted phenotype and tamoxifen efficacy are conflicting.[12, 23] Most studies were however underpowered to detect a difference between PMs and EMs.[15] A pooled analysis of all eligible patients treated with adjuvant tamoxifen is therefore imperative. In the TEAM study (**chapter 5**) and the ABCSG 8 study early breast cancer patients who were treated with 2 to 3 years of tamoxifen followed by an aromatase inhibitor were studied.[18] In these patient cohorts no association between CYP2D6 genotype and disease recurrence was found. These data may suggest that aromatase inhibitor use following tamoxifen negates or even reverses the higher likelihood of disease recurrence in patients with reduced CYP2D6 metabolism. Therefore, a pooled analysis of only tamoxifen treated early breast cancer patients, excluding those patients who subsequently used an aromatase inhibitor, will be of great value. Moreover, in this proposed meta-analysis, patients should only be included if sufficient CYP2D6 alleles were tested to avoid relevant misclassification and if the influence of LOH on genotype assignment was excluded in case DNA from tumor containing tissue was used. Preferably, data on concomitant CYP2D6 inhibitor use and tamoxifen adherence should be available as well.

The CYPTAM study (NTR1509; **chapter 11**) in early breast cancer and the CYPTAMBRUT-2 (NCT00965939) and ECOG E3108 (NCT01124695) in metastatic and locally advanced breast cancer will prospectively relate CYP2D6 predicted phenotype and endoxifen serum concentration to tamoxifen efficacy. The first results of the CYPTAM study are expected at the end of 2013 (3 years disease free survival). A positive association between endoxifen levels and clinical outcome is important as it may lead to the possibility of therapeutic drug monitoring. An additional positive association between CYP2D6 predicted phenotypes and tamoxifen efficacy may lead to a pre-treatment selection of those patients that we should withhold tamoxifen therapy or treat with a higher daily tamoxifen dose guided by endoxifen levels. The feasibility and safety of increasing endoxifen levels by increasing the tamoxifen dose was demonstrated in patients with an IM or PM phenotype (**chapters 8 and 9**). The tamoxifen dose was safely escalated from 20 to 40 mg (**chapter 8**) or even to a higher daily tamoxifen dose up to 120 mg (**chapter 9**). The latter dose escalation was guided by the steady state endoxifen levels measured before escalation. However, the long term risks and side effects of a tamoxifen dose higher than the registered 40 mg once daily dose are unknown. Such doses should not be prescribed until long term safety is ascertained.

In **chapter 10** a new 13C-dextrometorphan breath test (DM-BT) was equally predictive of endoxifen levels as the CYP2D6 genotype. However, a direct prospective correlation between DM-BT predicted CYP2D6 phenotype and tamoxifen efficacy is needed to explore its clinical relevance.

Genetic variants of metabolic enzymes other than CYP2D6 may be relevant in predicting tamoxifen efficacy and side effects. In addition, pharmacodynamic rather than pharmacokinetic markers may predict clinical outcome in tamoxifen treated breast cancer patients, such as variants of the estrogen receptor-1 gene (ESR1). The ESR1 encodes the estrogen receptor-α, which is the main target of tamoxifen and its active metabolites. In **chapters 5 and 6** we demonstrated that an increasing number of ESR1 PvuII C alleles was associated with worse disease free survival, but also that the same PvuII C allele in the CG haplotype was related to longer time to the first occurrence of hot flashes. These findings are interesting, but need to be validated in a prospective study, such as the CYPTAM study.

Tamoxifen response may also be related to the patient's adherence to the drug. Adherence is reported to be poor, ranging from 87% to 50% by year 4 of therapy.[31] In **chapter 7** we showed, to our knowledge for the first time, that poor tamoxifen adherence is associated with an increased risk of breast cancer events. Strategies directed toward improving tamoxifen adherence may improve recurrence-free and even overall survival. The efficacy of such strategies should be further explored.

If the results of the CYPTAM study and similar ongoing prospective studies will be that endoxifen is indeed predictive of tamoxifen efficacy, hormone receptor positive breast cancer patients may be best treated with a direct administration of endoxifen. Multiple clinical studies evaluating endoxifen are ongoing, including a study in which a novel formulation of endoxifen (endoxifen hydrochloride) is tested in patients with metastatic or locally recurrent estrogen receptor positive breast cancer (NCT01327781; NCT01273168). We are eagerly waiting for the results of these and subsequent similar studies.

Finally, recent data suggest that multiple other tamoxifen metabolites may contribute to its action in the treatment of breast cancer via aromatase inhibition. The tamoxifen metabolite with the strongest aromatase inhibition is the demethylated metabolite of endoxifen, norendoxifen.[32, 33] This again exemplifies that the tamoxifen metabolism is complex and needs to be further explored. We therefore may conclude that although tamoxifen is one of the oldest anticancer agents, there is still much about it to discover.

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